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Effect of Cucumber (*Cucumis sativus*) Fruit Homogenate on Hypotonicity–induced Haemolysis of Red Blood Cell

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Several findings and reports show that people with high intake of *Cucumis sativus* (Cucumber) have relief in pains, swelling and other inflammatory signs. Human red blood cell membrane stabilization has been used as a method to investigate the mechanism of action of anti-inflammatory drugs. The anti-inflammatory activity of **c**ucumber has been demonstrated in previous study. In this study, we aimed at assessing the effect of cucumber (*Cucumis sativus*) fruit homogenate on hypotonicity – induced haemolysis of red blood cell.
Methods: Whole fresh blood (3 ml) was collected from healthy volunteer into plastic tubes containing 0.1 volume of 3.8% trisodium citrate and used within 8 hr. The blood sample was centrifuged at 3000 x g for 10 min and the supernatant (plasma) discarded. The pellet was washed twice by resuspending in a volume of normal saline equal to the volume of the supernatant (plasma)

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and centrifuged at 3000 x g for 10 min. The pellet (0.1 ml) was resuspended in 2.5 ml of normal saline and used as the red blood cell (RBC).
Results: The results revealed that **c**ucumber (*Cucumis sativus*) fruit homogenate significantly ($p < 0.05$) inhibited hypotonicity-induced red blood cell haemolysis when compared to indomethacin (a known standard drug).
Conclusion: Cucumber has membrane stabilization effect on the red blood cell.

Keywords: Hypotonicity; membrane stabilization; red blood cells; inflammation; homogenate; Cucumis sativus.

1. INTRODUCTION

Red blood cells (RBCs) along with its membrane have always been a salient approach for the investigation of various physiological and metabolic characteristics [1]. During inflammation, the lysosomes lyse and release their component enzymes which produce a variety of disorders. Since human red blood cell membranes are similar to lysosomal membranes [2], human red blood cell membrane stabilization has, therefore**,** been used as a method to investigate the mechanism of action of anti-inflammatory drugs [3]. Stabilization of lysosomal membranes is key in limiting inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release [4]. Some non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin and acetylsalicylic acid are known to possess membrane stabilization properties [5,6,7] which may contribute to the potency of their anti-inflammatory effect [8,9].

Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and lysis, resulting in the release of haemoglobin, hence haemolysis. Haemolysis is an indication of the stability of red blood cell membrane [10,11]. The vitality of cells depends on the integrity of their membranes. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation. This notion is consistent with the observation that the breakdown of biomolecules leads to the formation of free radicals which in turn enhance cellular damage [12,13,14]. The progression of bone destruction seen in rheumatoid patient for example, has been shown to be due to increased free radical activity [15]. It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances [16].

Cucumis sativus (**c**ucumber) is a widely cultivated plant in the gourd family of Cucurbitaceae, which also includes important crops such as melon, water melon, and squash [17]. There is increased consumption of *Cucumis sativus* fruits possibly because of their high nutritional value. The nutritional composition of *Cucumis sativus* include protein, fat, and carbohydrate as primary metabolites; and dietary fibre which is important for the digestive system. *Cucumis sativus* contains some essential vitamins and antioxidants which are effective in human health [18]. The anti-inflammatory activity of **c**ucumber has been demonstrated in previous study [19]. In this study, we aimed to evaluate the *In vitro* membrane stabilization activities of *Cucumis sativus* fruit homogenate on hypotonicity – induced haemolysis of red blood cell (RBC).

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh whole *Cucumis sativus* L. fruits were purchased from Nsukka main market, Nsukka, Nigeria and were identified at the Bioresources Development and Conservation Programme Research Center, Nsukka, Nigeria by Mr Alfred Ozioko, an ethnobotanist of the herbarium unit with voucher number 13201400. The fruits were homogenized using high-speed blender and used without dilution [20].

2.2 Hypotonicity – Induced Haemolysis of Red Blood Cell of *C. sativus* Fruit

The effect of *Cucumis sativus* fruit homogenate on hypotonicity – induced haemolysis of red blood cell was investigated using the method of Murugesh [21] with minor modification.

Principle: Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and subsequently lysis. This results in the release of haemoglobins which absorbs maximally at 418 nm. Hence the optical density at 418 nm is reflection of haemoglobin concentration. Reflection of the stability of red blood cell membrane is thus measured by changes in optical density, changes in haemoglobin concentration in the medium.

Preparation of erythrocyte suspension: Fresh whole blood (3 ml) was collected from healthy human volunteer (after consenting to participate in the research, following the signing of the consent form and guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria), into plastic tubes containing 0.1 volume of 3.8% trisodium citrate and used within 8 hr. The blood sample was centrifuged at 3000 x g for 10 min and the supernatant (plasma) discarded. The pellet was washed twice by resuspending it in a volume of normal saline equal to the volume of the supernatant (plasma) and centrifuge at 3000 x g for 10 min. The pellet (0.1 ml) was resuspended in 2.5 ml of normal saline and used as the red blood cell (RBC).

Procedure: A set of seven tubes were used for the analysis. The reaction medium is shown in Table 1 below.

Table 1. Hypotonicity-induced haemolysis of RBC reaction medium

Tube	RBC (ml)	Distilled Water (ml)	Normal Saline (ml)	Fruit Homogenate (ml)
1	0.1	0	1.9	0
2	0.1	1.0	0.9	0
3	0.1	1.0	0.5	0.1
4	0.1	1.0	0.5	0.2
5	0.1	1.0	0.5	0.4
6	0.1	1.0	0.5	0.6
7	0.1	1.0	0.5	Indomethacin (0.4mg/ml)

The reaction medium was incubated at 37 ^{o}C for 1 hour. After incubation, each of the incubates was centrifuged at 3000 x g for 10 min to terminate the reaction. The absorptions of the respective supernatants were measured at 418 nm as a measure of extent of haemolysis. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde et al. [16].

$$\%\ \text{Inhibition of haemolysis} = 100 \times \frac{OD1 - OD2}{OD1}$$

Where:

OD1 = Optical density of hypotonic-buffered saline solution alone
OD2 **=** Optical density of test sample in hypotonic solution
Blank reaction medium contained 1.2ml normal saline and 0.8ml water.

3. RESULTS

Table 2 shows that the homogenate of *Cucumis sativus* fruits significantly inhibited lysis induced by water in dose dependent manner. When erythrocytes were suspended in water and later centrifuged, the supernatant was found to have a mean absorbance of 1.367 at 418 nm. On the other hand, suspension of the erythrocytes in normal saline, given the same treatment as in the case of water gave an absorbance of 0.280. The result showed that in the hypotonic (water) environment, there was liberation of haemoglobin and hence the high absorbance reading. Table 2 shows that when the homogenate was introduced, there was decreases in absorbance readings. This inhibition of haemolysis was found to be dose dependent, increasing with increased concentration of the extract in the medium. High percentage inhibition of haemolysis (64.9 and 94.4) was obtained at 0.4 and 0.6ml doses of the homogenate respectively, comparable to that of the standard drug, indomethacin (81.4).

4. DISCUSSION

Compounds with membrane-stabilizing effects are widely known for their abilities to inhibit the early phase of inflammation reactions [22,23]. The stabilization of the red blood cell membrane prevents the release of lytic enzymes and active mediators of inflammation, such as 5-hydroxytrptamine, histamine and kinins [24].

The homogenate of *Cucumis sativus* fruit was found to exhibit high membrane stabilization effect against hypotonicity induced haemolysis of the red cells as is shown by the level of inhibition of haemolysis. Protection against hypotonicity-induced haemolysis is related to membrane stabilization which is an anti-inflammatory index [22,25]. This inhibition of haemolysis was found to be dose dependent, increasing with increased amount of the homogenate in the medium and was comparable with that of indomethacin, a

Table 2. Inhibition of hypotonicity-induced haemolysis by the homogenate of *Cucumis sativus* fruit

Treatment	Mean O.D at 418 nm	% Inhibition of Haemolysis
Isotonic solution	0.140	-
Hypotonic solution (Control)	0.684	-
Test sample (0.1 ml)	0.610	10.8
Test sample (0.2 ml)	0.428	37.4
Test sample (0.4 ml)	0.240 [A]	64.9
Test sample (0.6 ml)	0.038 [A]	94.4
Indomethacin (0.4 mg/ml)	0.127 [A]	81.4

Mean values having upper case letters as superscripts down the column are considered significant ($p < 0.05$) compared to control. Percentage inhibition of haemolysis was calculated relative to control. n = 3

standard anti-inflammatory drug. Hypotonicity induced haemolysis of human red blood cells (HRBC) occurs due to water uptake by the cells and leads to the release of haemoglobin which absorbs maximally at 418 nm. Hence, the reduced optical density at 418 nm obtained for the various *Cucumis sativus* test samples was a reflection of the stabilization of the red cell membrane caused by the fruit homogenate. The fruit may also inhibit processes which stimulate or enhance the efflux of intracellular components. The erythrocyte membrane is analogous to the lysosomal membrane [26,27]. Its stabilization implies that *Cucumis sativus* may as well stabilize lysosomal membranes against the release of lytic enzymes.

Lysosomal enzymes play an important role in the development of acute and chronic inflammation. Most of the anti-inflammatory drugs exert their beneficial effects by either inhibiting the release of the enzymes or stabilizing the lysosomal membranes [26]. Stabilization of lysosomal membranes is important in preventing the leakage of serum protein and fluids into the tissue during the period of increased permeability caused by inflammatory mediators. The anti-haemolytic properties of *Cucumis sativus* fruit homogenate may be due to the presence of some active constituents such as flavonoids, tannins and saponins. It has been reported that flavonoids exert profound stabilizing effects on lysosomes both *in-vitro* and *in-vivo* in experimental animals [28,23] while tannins and saponins have the ability to bind cations and other biomolecules, and are able to stabilize the erythrocyte membrane [29]. The high membrane stabilizing activity of the homogenate of *Cucumis sativus* fruit observed in this study may be due to its flavonoids and tannins contents, which has been reported in previous studies [19,30].

5. CONCLUSION

The result of this study indicates that cucumber has the ability to inhibit hypotonicity – induced haemolysis of red blood cell, thereby confirming the membrane stability properties of cucumber.

CONSENT

As per international standards or university standards, respondents' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

All experimental protocols including the involvement of human participants were approved and followed the guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar P, Chaudhary N, Sharma NK, Maurya P.K. Detection of oxidative stress biomarkers in myricetin treated red blood cells. RSC Advances. 2016;6(102): 100028–100034.
2. Anosike CA, Onyechi Obidoa O, Ezeanyika LUS. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum)* Journal of Pharmaceutical Sciences. 2012;20(1): 76–81.
3. Amin C, Abdullah A, Shadman R, Shofiul A, Kishower SA. Human red blood cell membrane stability testing for the

estimation of anti-inflammatory activity of methanolic Extract of Millettia Pachycarpa Benth Leaves. International Journal of Pharmaceutical Sciences and Research. 2013;4(12):4587-4590

4. Sandhya S, Karunakar K, Ragavendhra P. HRBC Membrane Stabilization as a study tool to explore the Anti-Inflammatory activity of *Allium cepa* Linn.–Relevance for 3R. Journal of Advanced Medical and Dental Sciences Research. 2018;6(6):30-34.
5. Furst DE, Munster T. Non-steroidal anti-inflammatory drugs, disease-modifying anti-heumatic drugs, non-opoid analgesic and drugs used in gout. In: (B.G. Kartzung ed). Basic and Clinical Pharmacology 8th edn. Lange medical Books/McGraw-Hill, New York. 2001:596-623.
6. Mariappan G Saha BP, Sutharson L, Singh A, Garg S. Pandey L, Kumara D. Analgesic, anti-inflammatory, antipyretic and toxicological evaluation of some newer 3-methyl pyrazolone derivatives. Saudi Pharm J. 2011;19(2):115–122.
7. Sharma VK, Mamontov E, Tyagi M. Effects of NSAIDs on the nanoscopic dynamics of lipid membrane. Biochimica et Biophysica Acta (BBA) – Biomembranes. 2020;1862 (2):183100
8. Philip S, Chinedu I, Olawale O, Ismail MZ, Magaji AP. Effects of Ethanolic Extracts of Fruits of Dennettia tripetala on Kidney Function of Male Albino Rats. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2023;14(1):31–39. Available:https://doi.org/10.9734/ajbgmb/2023/v14i1306
9. Banerjee A, Manasa S, Ranganna G, Chowdhury S, Singh A, Ravindra NR, Raizada O, Chawl R. Unveiling the Rich Tapestry of Minor Fruit Crops: Cultivation Practices, Market Strategies, and Contributions to Agricultural Diversity and Sustainability. Journal of Advances in Biology & Biotechnology. 2024;27(5):821–834. Available:https://doi.org/10.9734/jabb/2024/v27i5844
10. Iwueke AV, Nwodo OFC, Okoli GO. Evaluation of the anti-inflammatory and analgesic activities of *Vitex doniana* leaves. African Journal of Biotechnology. 2006;5(20):1929-1935.
11. Sebastian H, Richard JA, Markus R, Laura H, Alexander D, Jose MM, Chris PV, Dawn MEB, Lars K, Christian W, Maikel CR. The Molecular Structure of Human Red Blood Cell Membranes from Highly Oriented, Solid Supported Multi- Lamellar Membranes, Scientific Report. 2017;7: 39661
12. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage *in-vivo* and in cell culture: How should you do it and what do the results mean? British Journal of Pharmacology. 2004;142(2): 231-225.
13. Lobo V, Patil A, Phatak AN, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118–126.
14. Alugoju Phaniendra, Dinesh Babu Jestadi, and Latha Periyasam. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem. 2015;30(1):11–26.
15. Pattison DJ, Silman AJ, Goodson NJ, Lunt M, Bunn D, Weich A, Bingham S, Khau KT, Day N, Symmons DPM. Vitamin C and the risk of developing inflammatory polyarthritis: Prospective case-control study. Annals of the Rheumatic Diseases. 2004;63:843-847.
16. Shinde UA, Phadke AS, Nair AM., Mungantiwan AA, Dikshirt VJ, Saref VO. Membrane stabilizing activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia.1999;70:251-257.
17. Chomicki, G Schaefer H, Renner SS. Origin and domestication of Cucurbitaceae crops: Insights from phylogenies, genomics and archaeology. New Phytologist. 2020:1240–1255.
18. Wang YH, Joobeur T, Dean RA, Staub JE. Cucurbits-Genome Mapping and Molecular Breeding in Plants 5. Vegetables. 2007:375.
19. Agatemor UM, Nwodo OFC, Anosike CA. Anti-inflammatory activity of *Cucumis sativus* L. British J. Pharm. Res. 2015; 8(2):1–8.
20. Zhu F, Du B, Xu B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Critical reviews in food science and nutrition. 2018;58(8):1260-70.
21. Murugesh N, Vember S, Damondaran C. Studies on erythrocyte membrane IV: *In-vitro* haemolytic activity of oleander extract. Toxicology Letters. 1981;8:33-38.
22. Hossain MM, Ahamed SK., Dewan SMR, Hassan MM, Istiaq A, Islam MS, Moghal

MMR. *In vivo* antipyretic, antiemetic, *in vitro* membrane stabilization, antimicrobial, and cytotoxic activities of different extracts from *Spilanthes paniculata* leaves. Biological Research. 2014;47(1):45.

23. Jameel MA, Gandasi RS, Praveen N, Biljo VJ, Fatima MA, Muneera QA. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022;27(9):2901
24. Saleem TKM, Azeem AK, Dilip, C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extacts of *Gendarussa vulgaris* Nees. Asian Pacific Journal of Tropical Biomedicine. 2011;1(2): 147–149.
25. Ojoghane E, Nwodo OFC. Comparison of extracts of *Cyphostemma glaucophilla* on total protein and membrane stabilization. Journal of Chemical and Pharmaceutical Research. 2010;2(4):31-37.
26. Mounnissamy VM, Kavimani S, Balu V, Drlin I, Quine S. Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Canjera rehedi.* Iranian. Journal of Pharmacology and Therapeutics. 2008;6:235-237
27. Douglas BA, Daniels K, Isaac TH, Martins E. Protective Effect of Bergapten against Human Erythrocyte Hemolysis and Protein Denaturation *In Vitro.* Int J Inflam. 2021;2021:1279359
28. David, S. Studies force new view on biology of flavonoids. Bio Medical. 2007; 541: 737-787.
29. Oyedapo OO. Biological activity of *Plyllanthus amarus* extracts on pragrow Dawley rats. Nigerian Journal of Biochemistry and Molecular Biology. 2001;16:83-86.
30. Agatemor UM, Nwodo OFC, Anosike CA. Phytochemical and proximate composition of cucumber (*Cucumis sativus*) fruit from Nsukka, Nigeria. African Journal of Biotechnology. 2018;17(38):1215-1219.

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